Communication:
Imported Platelets Demonstrate Decreased pH and Glucose by Reagent Strip Testing when Compared to Locally Derived Platelets

Pamela Clark,1,3 Theodore M. Parsons,1 James C. Boyd,1 Patti Dewey,3 and Paul D. Mintz1,2,3
Departments of Pathology1 and Internal Medicine2 and Medical Center Blood Bank,3
University of Virginia Health System, Charlottesville, Virginia

Abstract. The most common infectious risk from blood transfusion in the United States is bacterial contamination of platelet components. Although detection of bacterially contaminated platelet components is best achieved with a culture system, AABB standards permit alternatives including the use of staining methods or reagent strips. In this study, 13,216 consecutive platelet components were screened using reagent strips for evidence of the presence of bacteria. Testing was performed immediately prior to release of the platelet components for transfusion; 10,836 were collected locally and 2,380 were imported from other blood banks. A mix of whole-blood-derived and apheresis products was included. If either the glucose concentration was <250 mg/ml or the pH was <7.0, the platelet component was quarantined and a specimen was obtained for Gram’s stain and culture. Every transfused platelet component that was associated with a reported transfusion reaction was also tested by Gram’s stain and culture. Overall, 1.47% of imported platelet components were reactive while only 0.12% of locally collected platelets were reactive. Of 48 reactive platelet components, 44 were tested by Gram’s stain and culture. None was found to be bacterially contaminated. In summary, imported platelet components were significantly more likely to be falsely reactive by reagent strip screening as compared to locally prepared platelet components.

Keywords: platelets, reagent strips, bacterial contamination, bacteria detection, transfusion medicine

Introduction

Bacterial contamination of platelets has constituted the most common infectious risk of transfusion, with a previously estimated incidence of between one per thousand to one per three thousand platelet components [1-3]. Therefore, in March 2004, the American Association of Blood Banks (AABB) mandated that mechanisms be put in place to limit and detect the presence of bacteria in platelet components (AABB Standard 5.1.5.1) [4]. While bacterial culture is clearly the current method of choice for detecting bacterial contamination in single donor platelets, this method has not been practical for the evaluation of whole-blood-derived platelets, and the AABB has permitted the use of less sensitive techniques for detection of bacteria. Currently acceptable methods include bacterial staining and the use of urine multi-reagent strips to detect reduced pH and glucose concentration caused by bacterial growth [4].

Neither alternate technique for bacterial detection is ideal. While bacterial culture can routinely detect bacteria at levels of 10^2/ml, bacterial staining does not reliably yield a positive result until bacterial contamination reaches levels of 10^4 to 10^6/ml [2,5]. Bacterial staining procedures also require training and considerable expertise for accurate slide interpretation, and the staining procedures are not easily incorporated into the day-to-day operation of a hospital blood bank.
Using reagent strips, on the other hand, requires only that the technologist detect a color change on the strip after a short incubation period. This procedure can be easily performed immediately prior to pooling and does not require significant time for training prior to implementation [6]. However, reagent test strips are insensitive and require a bacterial concentration of at least $10^7$/ml before the results become positive [6,7]. Furthermore, positive results by reagent strip tests correlate poorly with actual bacterial contamination, since most positive test results cannot be confirmed by subsequent culture [8].

Therefore, prior to the implementation of AABB Standard 5.1.5.1 in March 2004, we elected to evaluate the reagent strip method because of its ease of use and ready incorporation into the day-to-day activities of a busy hospital blood bank. We were especially interested in determining how useful the procedure would be in detecting bacterially contaminated platelet products, thereby improving patient safety. We tested over 13,000 platelet products using reagent strips immediately prior to releasing the products from the blood bank for transfusion. Nearly all of the products that were deemed positive by the reagent strip tests were cultured. Further, all platelet products implicated in a transfusion reaction were also cultured in order to detect any positive units that were negative by dipstick methodology. Finally, to confirm the findings of our initial study, we used reagent strips to test an additional 16,800 platelet products.

Materials and Methods

Between October 2002 and September 2003 a total of 13,216 consecutive platelet components were tested for possible bacterial contamination immediately prior to release for transfusion by using reagent strips to determine the pH and glucose concentration (Multistix® 10 SG Reagent Strips, Bayer Corporation, Elkhart, IN). Of the platelet components, 10,836 were manufactured locally and 2,380 were imported by airplane from other blood centers. Of the locally manufactured products, 8,458 were whole-blood-derived and 2,378 were apheresis platelets. Of the imported products, 1,686 were whole-blood-derived and 694 were apheresis platelets.

The testing procedure was as follows: the platelet concentrate tubing was stripped into the platelet bag, followed by thorough mixing of the bag contents. The strippers were then released to allow the tubing to refill with platelet concentrate. Stripping and mixing were repeated once more. A one-inch segment of tubing was then heat-sealed and removed from the platelet bag for testing by the reagent strip. The end of the segment was cut off and one drop of platelet concentrate was squeezed onto the glucose color-block of the test strip. At exactly 30 sec, the glucose color-block was compared to the colors for glucose concentration provided on the Multistix® bottle and the results were recorded. For pH testing a drop of platelet concentrate was squeezed onto the pH color-block of the Multistix test strip. At exactly 60 sec, the pH color-block was compared to the colors for pH ranges provided on the Multistix bottle. Platelet components with an estimated glucose concentration <250 mg/dl or a pH <7.0 were quarantined, tested by Gram’s stain, and cultured. Additionally, every transfused platelet component associated with a reported transfusion reaction during this study was tested by Gram’s stain and cultured for bacteria.

From October 2003 through June 2005, 16,800 additional platelet products were tested for pH but not for glucose, using reagent strips as described above. In this study, only the import status of the platelet product was noted. No records were kept for whether the product was apheresis vs whole-blood-derived. The pH was only noted to be greater or less than 7.0; the actual pH value was not recorded.

For the first study, the effects of (a) strip positivity, (b) the category of platelet component preparation, and (c) the origin (local or imported) of platelet component preparation were examined by categorical data analysis using a main-effects linear model (CATMOD procedure, SAS). For the second study, the relationship between platelet component origin and strip positivity was assessed by contingency table analysis, using Fisher’s exact test.

Results

The results are given in Table 1. Forty-eight (0.36%) platelet components had pH values <7.0, of which 12 had glucose concentrations <250 mg/dl. No platelet component had a glucose concentration <250 mg/dl without a concomitant pH value <7.0. Thirty-four platelet components had a pH of 6.5, 6 had a pH of 6.0; for 8 platelet components the pH was reported as low, but the numerical result was not recorded. Overall, 35 of 2,380 (1.47%) imported platelet components had a reactive reagent strip test, while only 13 of 10,836 (0.12%) locally collected platelet components had a reactive test (p <0.001). Similarly, whole-blood-derived platelets were more likely than apheresis platelets to yield positive results by reagent strip test. Forty-seven of 10,144 (0.46%) whole-blood-derived platelet components were reactive, but only 1 of 3,072 (0.03%) apheresis platelet components tested positive by reagent strip (p = 0.0012).
Of 48 reactive platelet components, 44 were tested by Gram’s stain and cultured for bacteria. None was found to be bacterially contaminated. Additionally, Gram’s stains and bacterial cultures of 36 platelet components that were associated with reported transfusion reactions yielded consistently negative results.

Because glucose testing did not appear to detect bacterial contamination independently of pH, we tested an additional 16,800 platelets for pH only. Platelet products were not identified as to whether they were whole-blood-derived or apheresis platelets. In this second study, 62 of 2074 (2.99%) imported platelets tested positive, compared to 21 of 14,725 (0.14%) locally derived platelets (p <0.00001, Fisher’s exact test). The overall positive rate for all platelets was 0.49%. Of interest, our laboratory ceased receiving imported platelets toward the end of the study. The overall positive rate of reagent strip testing dropped to 0.17% once the platelets tested were all of local manufacture.

Discussion

We found that platelet components imported from other blood banks were significantly more likely to be falsely reactive by reagent strip testing as compared to locally prepared components. This is an important finding and suggests that the imported platelets were commonly subjected to conditions during shipment that reduced the pH to <7.0. The packaging of imported platelet components during shipment and the lack of agitation may impair release of carbon dioxide or decrease oxygen uptake, thereby decreasing the pH. Hunter et al [9] found that interruption of agitation of platelet concentrates for one day, either on the platelet agitator or within a stationary shipping container, caused diminution of pH due to increased lactate concentration, but did not significantly affect platelet function unless the pH dropped to <6.5. Glucose levels were found to decline in parallel with pH. In their study, a pH value <6.5 occurred only in products with high platelet counts of $1.2 - 3.5 \times 10^{12}$/L that were stored without agitation for 2 to 3 days [9].

Our findings are consistent with those of Hunter et al [9]. In our study, imported platelets were significantly more likely to have abnormally low pH levels, presumably due to a lack of agitation during shipment. Most (34 of 48 in our first study) had pH levels of 6.5 by reagent strip testing, indicating that the platelets likely remained functional despite low pH. While we did not perform platelet function studies, our standard operating procedure requires that technologists check for adequate swirling of all platelet products selected for transfusion. Therefore, all platelet products had passed this surrogate test for function prior to the reagent strip procedure.

Our findings are based on the largest number of reagent strip tests reported for platelet components. Since the study was designed to assess the use of reagent strips in the day-to-day hospital setting, technologists performed the test as part of the release procedure for platelet components. Therefore, the study indicates how well the reagent strip testing performs in its daily application in the blood bank rather than how well it performs in the research laboratory.

In our studies, the use of reagent strips did not identify any platelet products that were actually contaminated by bacteria. In their series of 3,093 platelet concentrates, Werch et al [8] reported that only 2 of 30 platelet concentrates that tested positive by reagent strips were positive by bacterial culture.

Table 1. Number and rate of reactive* platelets in locally collected vs imported platelet components.

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Locally collected components (n = 10,836)</th>
<th>Imported components (n = 2,380)</th>
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<tbody>
<tr>
<td></td>
<td>Number Reactive Non-Reactive</td>
<td>Number Reactive Non-Reactive</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>8,458 13 (0.15%)</td>
<td>1,686 34 (1.89%)</td>
</tr>
<tr>
<td>Apheresis</td>
<td>2,378 0 (0%)</td>
<td>694 1 (0.14%)</td>
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*Reactive signifies a pH <7.0 with or without a glucose concentration <250 mg/dl.
Our findings confirm that the positive predictive value of a reagent strip test is low. Because we did not perform bacterial cultures on all platelet components, the sensitivity and specificity of the reagent strip test cannot be calculated from our data, since the actual numbers of true-negative and false-negative tests are unknown. We did perform cultures on platelet products implicated in transfusion reactions to determine whether such products might have infected patients despite negative reagent strip testing and therefore would represent false negative results. We found no such instances of bacterial contamination.

While more sensitive and specific methods for detecting bacterial contamination of whole blood derived platelets would clearly be preferable, the reagent strip method is easy to use and easy to incorporate into daily blood bank operations. It should not result in significant product loss owing to false positive results unless a large proportion of platelet components are imported. The interdiction of even one contaminated platelet component by reagent strip testing would make the procedure worthwhile until more reliable, cost effective techniques are developed for routine use.

References